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Classification of Subjects as Slow or Rapid Inactivators of Isoniazid Based on the Ratio of Acetylisoniazid to Isoniazid in Urine Determined by a Simple Colorimetric Method

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A method for classifying subjects as slow or rapid inactivators of isoniazid based on the ratio of acetylisoniazid to isoniazid in a 3-4 hour urine collection following an intramuscular dose of isoniazid 3 mg./kg. body-weight has been described. Isoniazid and acetylisoniazid have been estimated using methods requiring the use of only a photo-electric colorimeter. Of the 279 patients investigated, 169 (61 per cent) were classified as slow inactivators and 110 (39 per cent) as rapid inactivators. This classification is an excellent agreement (97 per cent) with that based on a standard spectrophotometric method.

Introduction

Earlier studies at the Tuberculosis Chemotherapy Centre had demonstrated that patients can be conveniently classified as slow or rapid inactivators of isoniazid on the basis of the ratio of acetylisoniazid to isoniazid (A/I ratio) in a 3-4 hour urine collection following an intramuscular dose of isoniazid 3 mg./kg. body-weight (Venkataraman *et al.*, 1972; Sarma *et al.*, 1974). Estimation of isoniazid by these methods required the use of spectrophotometer which is not readily available in most laboratories in the developing countries. The present study sought to evaluate the use of a photo-electric colorimeter for the estimation of both isoniazid and acetylisoniazid and assess the efficiency of the resultant A/I ratio for purposes of classification in comparison with a standard spectrophotometric method (Sarma *et al.*, 1974). The availability of a simple method requiring the use of only a photo-electric colorimeter would make it feasible to adopt once-weekly chemotherapy for slow inactivators and twice-weekly chemotherapy for rapid inactivators (Tuberculosis Chemotherapy Centre, Madras, 1970; 1973).

Material and Methods

Two hundred eighty one patients with a mean body-weight of 39.6 kg. (95 per cent range 28.0-51.6 kg.) were administered an intramuscular dose of isoniazid 3 mg./kg.

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body-weight, after verifying that the urine was negative for acetylisoniazid by the qualitative test of Eidus and Hamilton (1964). The total urine excreted over the period 3-4 hours was collected and a sample stored at –20°C for a period not exceeding one week. Two samples that were positive for sugar by the Benedict's test were excluded, because it is known that estimates of acetylisoniazid by the colorimetric method, which is basically the same as the method of Venkataraman *et al.* (1968), would be inaccurate in the presence of sugar in the urine (Sarma *et al.*, 1974). The A/I ratios were determined for the remaining 279 samples by the colorimetric method as well as a standard spectrophotometric method, after separate coding and randomisation.

Calorimetric method : Acetylisoniazid was estimated by the method of Venkataraman *et al.* (1968), without prior oxidation using potassium permanganate or the reverse blank procedure. For recording the absorption, a photo-electric colorimeter (Systronits–Type 101) with filter No. 625 (wave length range : 510-590 mu) wasemployed, instead of a spectrophotometer.

Isoniazid was estimated using a slight modification of the procedure described by Ellard *et al.* (1973). 1.0 ml. of urine was treated with 0.1 ml. of 2N hydrochloric acid and left at room temperature for 15 minutes. After neutralisation with 0.1 ml. of 2N sodium hydroxide solution, 3.0 ml. of a solution of dipotassium hydrogen phosphate (34 g./100 ml.) was added, followed by 1.0 ml. of freshly prepared 0.5 per cent aqueous picryl sulphonic acid solution. 6.0 ml. of isobutyl methyl ketone were added, the contents were left in the dark for 15 minutes and then shaken thoroughly by hand, and the organic and aqueous layers were allowed to separate. The absorption of the organic layer was measured with the photo-electric colorimeter using filter No. 623 (wave length range : 460-540 mu). Urine samples giving readings greater than those given by 40 µg./ml. isoniazid were appropriately diluted prior to reaction and the above procedure was repeated. A reagent blank and suitable isoniazid standards (5 and 10 µg. /ml.) were set up each day using 1 : 4 diluted normal urine.

Spectrophotometric method : Acetylisoniazid and isoniazid were estimated by the extraction methods of Sarma *et al.* (1974) and Rao *et al.* (1971), respectively.

Results

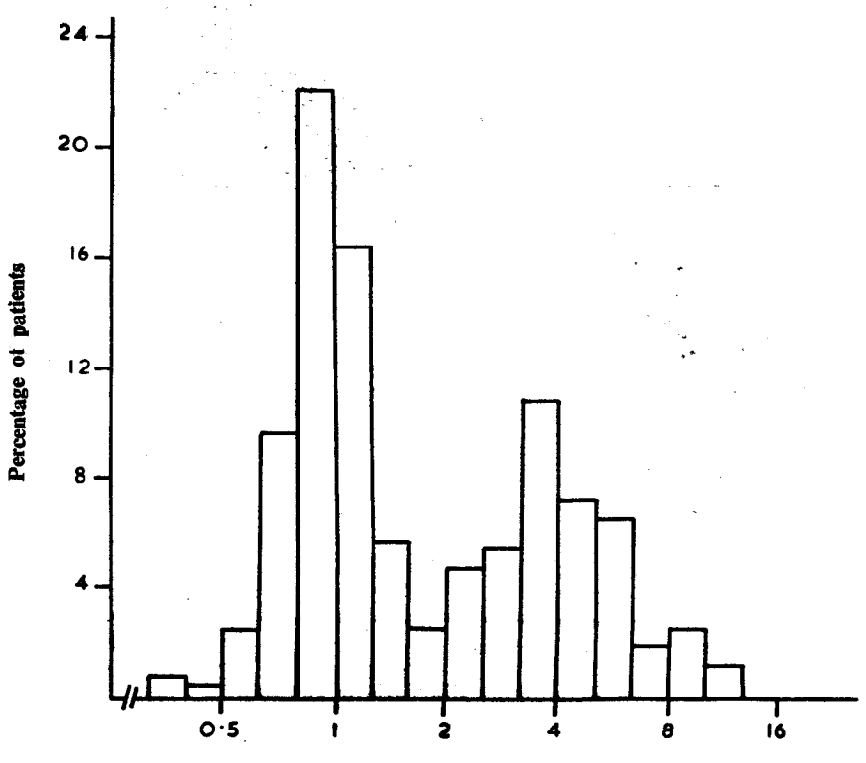
Correlation between estimates by colorimetric and spectrophotometric methods : The correlation between estimates by the colorimetric method and the spectrophotometric method was very high, both for isoniazid (r=+0.97) and for acetylisoniazid (r= + 0.98). In the case of isoniazid, the estimate were also very similar, the mean values. being 49 µg./ml. with the colorimetric method and 46 µg./ml., with the spectrophotometric method. In the case of acetylisoniazid, however, estimates by the colorimetric method were consistently higher by 47 per cent on an average due to the contribution of the hydrazones (Sarma *et al.*, 1974).

Classification of patients as slow or rapid inactivators of isoniazid : With the colorimetric method, the distribution of the 279 patients according to the A/I ratio (on a

logarithmic scale) was clearly bimodel (Graph 1). Based on this histogram, patients with a ratio of 2.00 or more were classified as rapid inactivators (110 patients) and those with a ratio of 1.99 or less as slow inactivators (169 patients).

Graph 1

Distribution of 279 patients according to the colorimetric determination of the ratio of acetylisoniazid to isoniazid in urine, 3-4 hours after an intramuscular dose of isoniazid 3 mg./kg. body-weight.

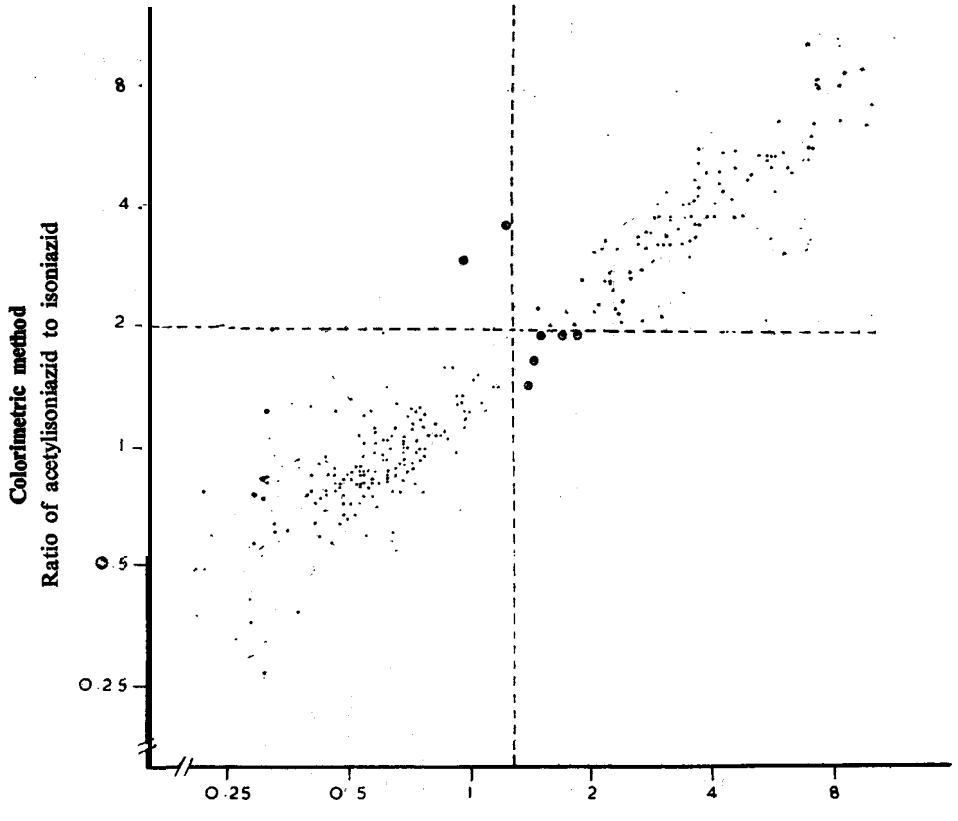


The distribution of the A/I ratios by the spectrophotometric method suggested the criterion for a rapid inactivator to be a ratio of 1.25 or more.

The classification of 272 (97 per cent) of the 279 patients was identical by the two methods (Graph 2); there was a disagreement in the remaining 7 patients, 5 of whom were classified as slow inactivators and 2 as rapid inactivators by the colorimetric method, Six of these 7 patients had their rate of inactivation determined on another occasion by one of two other methods (Tuberculosis Chemotherapy Centre, Madras, 1973; Sarma ***et al.***, 1976., in all 6 patients, the classification by the standard spectrophotometric method was confirmed.

Graph 2

Correlation between the A/I ratios in urine by the spectrophotometric and colorimetric methods.



Discussion

Photo-electric colorimeters can be used for recording the extinctions of solutions whose absorption maxima lie in the visible range of the electromagnetic spectrum (between 400 and 800 mu). In the methods described previously (Venkataraman *et al.*, 1972; Sarma *et al.*, 1974), isoniazid was estimated following condensation with vanillin. The isoniazid-vanillin condensation product has an absorption maximum at 380 mu and a spectrophotometer is therefore essential for its estimation. In the present paper, isoniazid has been estimated following condensation with picryl sulphonic acid. The isoniazid-picryl sulphonic acid condensation product has an absorption maximum at 500 mu; it was therefore possible to use a photo-electric colorimeter to record the optical density.

The findings of the present study, involving the determination of A/I ratios in the 3-4 hour urine collection following an intramuscular dose of isoniazid 3 mg./kg. body-weight in 279 patients, indicate that the colorimetric method can efficiently classify patients as slow or rapid inactivators of isoniazid. Thus, the proportion of correct classifications, in comparison with a standard spectrophotometric method, was 97 per cent. The colorimetric method cannot be applied to urine samples containing sugar, but this may not be a serious limitation as the proportion of patients with glycosuria is seldom high, Krishnaswami (1960) reported this proportion to be 1.8 per cent in 1629 tuberculous patients admitted to the Government Tuberculosis Sanatorium at Madras.

Based on a follow-up investigation in 559 patients conducted at the Tuberculosis Chemotherapy Centre, Madras with the method of Venkataraman et al. (1972) there is evidence (unpublished) that a 2-3 hour urine collection discriminates between slow and rapid inactivators as efficiently as a 3-4 hour collection, but that a O-2 hour collection is unsatisfactory. Therefore, in laboratories where facilities for giving injections are available, a 2-3 hour collection may be obtained and the A/I ratio determined by the colorimetric method. Fresh. criteria would, however, have to be derived.

Oral administration of isoniazid is generally more applicable and acceptable than intramuscular administration. A classification procedure using a uniform oral dose of isoniazid 300 mg. and determination of the A/I ratio in a 5-6 hour urine collection has recently been reported from this Centre (Sarma *et al.,* 1976). This would constitute a simple test, especially if the estimations of isoniazid and acetylisoniazid are made by the colorimetric method.

Eidus *et al.* (1973) using oral administration of isoniazid, and Rao *et al.* (1970) and Viznerova *et al.* (1973) using oral administration of sulphadimidine, have described methods involving urine collection for the classification of patients as slow or rapid inactivators of isoniazid. The procedure described by Eidus *et al.* (1973) is simple, as only acetylisoniazid is estimated in the urine sample (before and after acetylation with acetic anhydride) using a photo-electric colorimeter. However, the dose administered was weight-dependent (10 mg./kg.) and a fairly late hour urine collection (6-8 hours) was employed. Methods using sulphadimidine also employ weight-dependent dose. However, they require the estimation of only one component viz., sulphadimidine, before and after acid hydrolysis of the urine sample, and can be readily adapted for use with a photo-electric colorimeter. Further, both sulphadimidine and acetylsulphadimidine are more stable than isoniazid and acetylisoniazid, an important factor for consideration under field conditions.

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